iGEM TU/e 2014
Biomedical Engineering

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Site Directed Mutagenesis

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Title
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1 PCR

Component	Quantity/mass/final concentration	Volume (uL)
H2O	To 25 µL	..
10× QuikChange Lightning Multi reaction buffer	1x	2.5 µL
Plasmid DNA	100 ng (stock … ng/uL)	..
Forward primer	100 ng (10 uM stock)	
dNTP mix	1x	1 µL
QuikChange Lightning Multi enzyme blend	1x	1 µL
Total		25 µL

Segment	Cycles	Temperature	Time
1	1	95°C	2 min
2	30	95 °C	20 sec
		55 °C	30
		65 °C	30 seconds/kb of plasmid length -> 3 min
3	1	65 °C	5 minutes

2 Dpn I Digestion of the Amplification Products

- Add 1 µL of Dpn I restriction enzyme directly to each amplificationreaction.
- Gently and thoroughly mix each reaction mixture by pipetting the solution up and down several times. Spin down the reaction mixtures in a microcentrifuge for 1 minute, then immediately incubate each reaction at 37°C for 5 minutes to digest the parental (nonmutated) ds-DNA.

3 Transformation of XL10-Gold Ultracompetent Cells

Note Please see Transformation Guidelines for detailed information about parameters that affect transformation of XL10-Gold ultracompetent cells. XL10-Gold cells are resistant to tetracycline and chloramphenicol. If the mutagenized plasmid contains only the tetR or the camR resistance marker, an alternative strain of competent cells must be used.

- Gently thaw the XL10-Gold ultracompetent cells on ice. For each mutagenesis reaction, aliquot 45 µL of the ultracompetent cells to a prechilled 14-ml BD Falcon polypropylene round-bottom tube.

- Add 2 µL of the β -ME mix provided with the kit to the 45 µL of cells. Using an alternative source of β-ME may reduce transformation efficiency.
- Swirl the contents of the tube gently. Incubate the cells on ice for 10 minutes, swirling gently every 2 minutes.
- Transfer 4 µL of the Dpn I-treated DNA from each mutagenesis reaction to a separate aliquot of the ultracompetent cells.
- Swirl the transformation reactions gently to mix, then incubate the reactions on ice for 30 minutes.
- Preheat SOC (see Preparation of Media and Reagents) in a 42°C water bath for use in step 9.
- Heat-pulse the tubes in a 42°C water bath for 30 seconds. The duration of the heat pulse is critical for obtaining the highest efficiencies. Do not exceed 42°C.
- Incubate the tubes on ice for 2 minutes.
- Add 0.5 ml of preheated (42°C) SOC to each tube and incubate the tubes at 37°C for 1 hour with shaking at 225–250 rpm.
- Plate 250 µL of each transformation reaction
- Incubate the transformation plates at 37°C for >16 hours.